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20427 U.S. PTO

ATTORNEY DOCKET NO. 22000.0129U1

Page 1 of 4

**PROVISIONAL APPLICATION FOR PATENT
COVER SHEET**22154 U.S. PTO
60/550037This is a request for filing a **PROVISIONAL APPLICATION FOR PATENT** under 37 C.F.R. § 1.53(c).

Docket Number		22000.		Type a Plus Sign (+) inside this box		+	
INVENTOR(s)							
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (City and Either State or Foreign Country)				
Hawiber	Jacek		1836 Laurel Ridge Dr., Nashville, TN 37215				
TITLE OF INVENTION (500 characters max)							
CELL-PERMEABLE SOCS PROTEIN THAT INHIBIT CYTOKINE-INDUCED SIGNALING							
CORRESPONDENCE ADDRESS							
Lizette M. Fernandez, Ph.D. Customer Number 23859							
ENCLOSED APPLICATION PARTS (Check All That Apply)							
<input checked="" type="checkbox"/> Provisional Application Title Page <i>Number of Pages</i> [01]							
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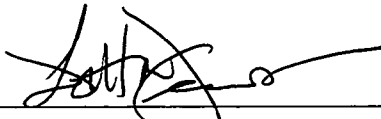
- ☒ Applicant claims small entity status. See 37 CFR § 1.27.
- ☒ A Credit Card Payment Form PTO-2038 is enclosed to cover the filing fees.
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Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
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Hawiger, J.)
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Application No. Unassigned)
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Filing Date: Concurrently)
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For: CELL-PERMEABLE SOCS PROTEINS)
THAT INHIBIT CYTOKINE-INDUCED)
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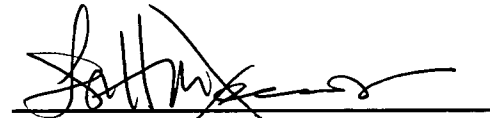
Pursuant to 37 C.F.R. § 1.136(a)(3), the Commissioner is hereby requested and authorized to treat any concurrent or future reply in the above-identified application, requiring a petition for an extension of time for its timely submission, as incorporating a petition for extension of time for the appropriate length of time.

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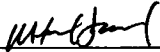


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Michael Laird

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Attorney Docket No. 22000.0129U1

UTILITY PATENT - PROVISIONAL FILING

PROVISIONAL APPLICATION FOR LETTERS PATENT

TO ALL WHOM IT MAY CONCERN:

Be it known that I, JAECK HAWIGER, residing respectively at 1836 Laurel Ridge Dr., Nashville, TN 37215 have invented new and useful improvements in

**CELL-PERMEABLE SOCS PROTEINS THAT INHIBIT
CYTOKINE-INDUCED SIGNALING**

for which the following is a specification.

**CELL-PERMEABLE SOCS PROTEINS THAT INHIBIT CYTOKINE-
INDUCED SIGNALING**

5 Staphylococcal enterotoxin B (SEB) listed by the U.S. Public Health Service as
a potential bioweapon is an extremely potent inducer of toxic shock syndrome. SEB
forms intercellular synapses between major histocompatibility complex (MHC)-Class II
and T cell receptor (TCR) that induce robust production of inflammatory cytokines
such as TNF- α , IFN- γ , and IL-6. TNF- α and IFN- γ were shown to be responsible for
10 inducing acute tissue injury and collapse of vascular system (toxic shock syndrome)
caused by SEB. To inhibit IFN- γ -induced signal transduction pathway, cell-permeable
suppressor of cytokine signaling (SOCS) proteins that contain a membrane-
translocating motif (MTM) originated from a signal sequence hydrophobic region of
FGF-4 were designed. These proteins inhibit IFN- γ -induced signaling in cultured cells
15 as well as in primary cells. Thus, the compositions disclosed herein be utilized to

 The present invention provides a method for inhibiting a cytokine-induced
response in a cell, comprising administering to the cell a complex comprising a cell
membrane permeable hydrophobic region of a signal peptide linked to a polypeptide
comprising an amino acid sequence encoding a SOCS protein or a fragment thereof.
20 Examples of the complexes that can be utilized in the methods of the present invention
are set forth in the Examples herein.

 The present invention also provides a method for inhibiting a cytokine-induced
response in a cell in a subject, comprising administering to the cell in a subject a
complex comprising a cell membrane permeable hydrophobic region of a signal peptide
25 linked to a polypeptide comprising an amino acid sequence encoding a SOCS protein
or a fragment thereof. Also provided by the present invention provides a method for
treating or preventing a cytokine-induced inflammatory response in a subject,
comprising administering to the subject a complex comprising a cell membrane
permeable hydrophobic region of a signal peptide linked to a polypeptide comprising
30 an amino acid sequence encoding a SOCS protein or a fragment thereof. The
inflammatory response or inflammation in the subject can be a systemic inflammatory
response or a cellular inflammatory response. The cytokines involved in the
inflammatory response can be, but are not limited to, TNF- α , IFN- γ , and IL-6. These
pathways are well known in the art and one of skill in the art would know how to

conduct assays that measure the effects of an SOCS protein of this invention on molecules involved in cytokine pathways, such as TNF- α , IFN- γ , and IL-6 pathways.

5 The methods and peptides of the invention can also be used to treat or prevent inflammatory responses that affect the function of specific organs or organ systems, for example, but not limited to, the liver, bowel, kidney, joints, skin, pancreas, central nervous system, peripheral nervous system, bladder, or reproductive organs. In some cases, the inflammatory response is caused by an inflammatory disease, for example, an autoimmune disease. Examples of such autoimmune diseases include, but are not limited to, inflammatory bowel disease, Crohn's disease, glomerulonephritis, multiple sclerosis, lupus erythematosus, rheumatoid arthritis, psoriasis, or juvenile diabetes. The methods and peptides of the invention can also be used to treat chronic or acute inflammatory diseases and conditions of the skin, for example, psoriasis, eczema, or contact dermatitis.

15 The methods of the present invention can be utilized to inhibit IFN- γ induced signaling *in vitro*, *ex vivo* and *in vivo*. Suitable import conditions for the compositions of the present invention are exemplified herein and include cell and complex temperature between about 180°C and about 42°C, with a preferred temperature being between about 22°C and about 37°C. For administration to a cell in a subject the complex, once in the subject, will of course adjust to the subject's body temperature. 20 For *ex vivo* administration, the complex can be administered by any standard methods that would maintain viability of the cells, such as by adding it to culture medium (appropriate for the target cells) and adding this medium directly to the cells. As is known in the art, any medium used in this method can be aqueous and non-toxic so as not to render the cells non-viable. In addition, it can contain standard nutrients for 25 maintaining viability of cells, if desired. For *in vivo* administration, the complex can be added to, for example, a blood sample or a tissue sample from the patient or to a pharmaceutically acceptable carrier, e.g., saline and buffered saline, and administered by any of several means known in the art. Examples of administration include parenteral administration, e.g., by intravenous injection including regional perfusion. 30 through a blood vessel supplying the tissues(s) or organ(s) having the target cell(s), or by inhalation of an aerosol, subcutaneous or intramuscular injection, topical administration such as to skin wounds and lesions, direct transfection into, e.g., bone marrow cells prepared for transplantation and subsequent transplantation into the

subject, and direct transfection into an organ that is subsequently transplanted into the subject. Further administration methods include oral administration, particularly when the complex is encapsulated, or rectal administration, particularly when the complex is in suppository form. A pharmaceutically acceptable carrier includes any material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected complex without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is administered. Administration can be performed for a time length of about 1 minute to about 72 hours. Preferable time lengths are about 5 minutes to about 48 hours, and even more preferably about 5 minutes to about 20 hours, and even more preferably about 5 minutes to about 2 hours. Optimal time lengths and conditions for any specific complex and any specific target cell can readily be determined, given the teachings herein and knowledge in the art. Specifically, if a particular cell type *in vivo* is to be targeted, for example, by regional perfusion of an organ or tumor, cells from the target tissue can be biopsied and optimal dosages for import of the complex into that tissue can be determined *in vitro*, as described herein and as known in the art, to optimize the *in vivo* dosage, including concentration and time length. Alternatively, culture cells of the same cell type can also be used to optimize the dosage for the target cells *in vivo*.

For either *ex vivo* or *in vivo* use, the complex can be administered at any effective concentration. An effective concentration is that amount that results in importation of the biologically active molecule into the cell. Such a concentration will typically be between about 0.5 nM to about 100 μ M (culture medium concentration (*ex vivo*) or blood serum concentration (*in vivo*)). Optimal concentrations for a particular complex and/or a particular target cell can be readily determined following the teachings herein. Thus, *in vivo* dosages of the complex include those which will cause the blood serum concentration of the complex to be about 0.5 nM to about 100 μ M. A preferable concentration is about 2 nM to about 50 μ M. The amount of the complex administered will, of course, depend upon the subject being treated, the subject's age and weight, the manner of administration, and the judgment of the skilled administrator. The exact amount of the complex will further depend upon the general condition of the subject, the severity of the disease/condition being treated by the administration and the particular complex chosen. However, an appropriate amount can

be determined by one of ordinary skill in the art using routine optimization given the teachings herein.

Parenteral administration, e.g., regional perfusion, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, such as liquid solutions, suspensions, or emulsions. A slow release or sustained release system, such as disclosed in U.S. Patent No. 3,710,795, can also be used, allowing the maintenance of a constant level of dosage.

Depending on the intended mode of administration (e.g., but not limited to, intravenous, parenteral, transcutaneous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, intrarectal, intravaginal, aerosol, or oral), the pharmaceutical compositions may be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, lotions, creams, gels, or the like, preferably in unit dosage form suitable for single administration of a precise dosage. The compositions will include, as noted above, an effective amount of the selected drug in combination with a pharmaceutically acceptable carrier and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc.

For solid compositions, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc. an active compound as described herein, and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences.

The present invention provides a complex comprising a suppressor of cytokine signaling (SOCS) protein or a fragment thereof linked to an importation competent signal peptide or cell membrane-permeable hydrophobic region of a signal peptide. Any SOCS protein, such as SOCS-1, SOCS-2 or SOCS-3 (or fragment thereof), from

any species, can be used, with its selection being dependent upon the purpose to be accomplished by importing the molecule into the selected cell.

An example of a full-length SOCS-1 amino acid sequence is provided herein as SEQ ID NO: 3. Nucleic acid sequences encoding this amino acid sequence are also
5 provided herein. The amino acid sequence of human SOCS-1 as well as a nucleic acid encoding human SOCS-1 can be accessed via GenBank under Accession No. NM_003745. The sequences and all information disclosed under Accession No. NM_003745 are incorporated herein in their entirety by this reference. The amino acid
10 sequence of mouse SOCS-1 as well as a nucleic acid encoding mouse SOCS-1 can be accessed via GenBank under Accession No. NM_009896. The sequences and all information disclosed under Accession No. NM_009896 are incorporated herein in their entirety by this reference.

The amino acid sequence of human SOCS-2 as well as a nucleic acid encoding human SOCS-2 can be accessed via GenBank under Accession No. NM_003877. The
15 sequences and all information disclosed under Accession No. NM_003877 are incorporated herein in their entirety by this reference. The amino acid sequence of mouse SOCS-2 as well as a nucleic acid encoding mouse SOCS-2 can be accessed via GenBank under Accession No. NM_007706. The sequences and all information
20 disclosed under Accession No. NM_007706 are incorporated herein in their entirety by this reference.

An example of a full-length SOCS-3 amino acid sequence is provided herein as SEQ ID NO: 4. Nucleic acid sequences encoding this amino acid sequence are also
provided herein. The amino acid sequence of human SOCS 3 as well as a nucleic acid encoding human SOCS-3 can be accessed via GenBank under Accession No.
25 NM_003955. The sequences and all information disclosed under Accession No. NM_003955 are incorporated herein in their entirety by this reference. The amino acid sequence of mouse SOCS-3 as well as a nucleic acid encoding mouse SOCS-3 can be accessed via GenBank under Accession No. NM_007707. The sequences and all
30 information disclosed under Accession No. NM_007707 are incorporated herein in their entirety by this reference. Fragment of the sequences disclosed herein can be combined with an appropriate signal peptide to make the SOCS polypeptides of the present invention.

An "importation competent signal peptide" or "cell membrane-permeable hydrophobic region of a signal peptide" as used herein, is a sequence of amino acids

generally of a length of about 10 to about 50 or more amino acid residues, many (typically about 55-60%) residues of which are hydrophobic such that they have a hydrophobic, lipid-soluble portion. The hydrophobic portion is a common, major motif of the signal peptide, and it is often a central part of the signal peptide of protein
5 secreted from cells. A signal peptide is a peptide capable of penetrating through the cell membrane to allow the export of cellular proteins. The signal peptides of this invention, are also "importation competent" or "cell-permeable," i.e., capable of penetrating through the cell membrane from outside the cell to the interior of the cell. The amino acid residues can be mutated and/or modified (i.e., to form mimetics) so
10 long as the modifications do not affect the translocation-mediating function of the peptide. Thus the word "peptide" includes mimetics and the word "amino acid" includes modified amino acids, as used herein, unusual amino acids, and D-form amino acids. All importation competent signal peptides encompassed by this invention have the function of mediating translocation across a cell membrane from outside the cell to
15 the interior of the cell. Such importation competent signal peptides could potentially be modified such that they lose the ability to export a protein but maintain the ability to import molecules into the cell.

Signal peptides can be selected, for example, from the SIGPEP database, which also lists the origin of the signal peptide. When a specific cell type is to be targeted, a
20 signal peptide used by that cell type can be chosen. For example, signal peptides encoded by a particular oncogene can be selected for use in targeting cells in which the oncogene is expressed. Additionally, signal peptides endogenous to the cell type can be chosen for importing biologically active molecules into that cell type. And again, any selected signal peptide can be routinely tested for the ability to translocate across
25 the cell membrane of any given cell type according to the teachings herein. Specifically, the signal peptide of choice can be conjugated to a SOCS protein and administered to a cell, and the cell is subsequently screened for the presence of the active molecule. In the methods of the present invention, the hydrophobic region of a signal peptide can be the membrane translocating motif of FGF-4. For example, the
30 MTM can be the amino acid sequence provided herein as SEQ ID NO: 2 (AAVLLPVLLAAP). SEQ ID NO: 3 is the MTM utilized, as described in the Examples, to make SOCS-1 and SOCS-3 fusion proteins.

The amino acid sequence of the full-length human FGF-4 and the human FGF-4 signal sequence, as well as sequences encoding human FGF-4 and its signal sequence

can be accessed via GenBank under Accession Nos. NM_002007 and NP_001998. The sequences and all information disclosed under Accession Nos. NM_002007 and NP_001998 are incorporated herein in their entirety by this reference. The amino acid sequence of the full-length mouse FGF-4 and the mouse FGF-4 signal sequence, as
5 well as sequences encoding mouse FGF-4 and its signal sequence can be accessed via GenBank under Accession No. NM_010202.

EXAMPLES

10 SOCS Fusion Proteins

The present invention provides several SOCS fusion proteins. For example, the present invention provides a complex or a fusion protein comprising a histidine tag, a SOCS1 polypeptide and a MTM (SEQ ID NO: 5). Also provided by the present
15 invention is a complex or fusion protein comprising a histidine tag, a MTM and a SOCS1 polypeptide (SEQ ID NO: 6). Therefore, the present invention provides SOCS-1 fusion proteins that are flanked at the NH2 terminus by MTM (SEQ ID NO: 6) and a SOCS-1 fusion that is flanked at the COOH terminus by MTM (SEQ ID NO: 5). SOCS-1 proteins that bear MTM at either end of the sequence (SEQ ID NO: 5 and SEQ
20 ID NO: 6) can be utilized to inhibit IL-6 signaling and IFN- γ induced signaling.

The present invention also provides the full-length SOCS-3 protein with a histidine tag (SEQ ID NO: 7). Also provided is a complex or fusion protein comprising a histidine tag, a SOCS-3 polypeptide and a MTM (SEQ ID NO: 8). Further provided by the present invention is a complex or a fusion protein comprising a histidine tag, a
25 MTM and a SOCS-3 polypeptide (SEQ ID NO: 9). Therefore, the present invention provides SOCS-3 fusion proteins that are flanked at the NH2 terminus by MTM (SEQ ID NO: 9) and a SOCS-1 fusion that is flanked at the COOH terminus by MTM (SEQ ID NO: 8). As described below, the SOCS3 protein with MTM (SEQ ID NO: 7) was inactive in terms of inhibition of STAT1 phosphorylation. In striking contrast to an
30 MTM-deficient protein, two recombinant SOCS-3 proteins that bear MTM at either end of the sequence (SEQ ID NO: 8 and SEQ ID NO: 9) had significant inhibitory effects on STAT1 phosphorylation. Thus, these polypeptides are examples of SOCS-3 fusion proteins that can be utilized to inhibit IL-6 induced signaling and inhibit IFN- γ induced signaling.

Nucleic acid sequences encoding SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 9 are also provided herein. The SOCS fusion proteins described herein can also be utilized without a histidine tag in the methods of inhibiting signaling in
5 cells (*in vitro*, *ex vivo* and *in vivo*) or an inflammatory response in a subject.

C.4. Development of SOCS3-Derived Cell-Permeant Functional Inhibitor of Intracellular Signaling.

We succeeded in producing cell permeant SOCS3 protein, to be used as an inhibitor of IL6-induced signaling. SOCS3 is a modular, SH₂-containing, 225 amino acids-long protein. It was enabled by us to cross the plasma membrane of macrophages and inhibit Interferon γ -induced signaling.

C.4.1. Preparation of recombinant SOCS-3 Proteins. Recombinant SOCS-3 proteins were constructed without and with Membrane Translocating Motif (MTM) by amplifying SOCS sequences. The PCR product was cleaved with *NdeI* and cloned into the *NdeI* site of 6 histidine tagged expression vector (pET-28a(+)). The resulting plasmids were used to express SOCS3 protein under the control of the *lacI* promoter in *E. coli* strain BL21-CodonPlus (DE3). The recombinant fusion proteins were purified under denaturing condition by chromatography on nickel-nitrilotriacetic acid (Ni-NTA) meta-affinity beads as directed by the supplier (Qiagen). Purified proteins were refolded, concentrated and dissolved in DMEM. Three recombinant murine SOCS 3 proteins, denoted HS3, HS3M, and HMS3, were produced and purified (Figs. 12 and 13). They contained a full length SOCS 3 sequence flanked at the NH₂ terminus by polyhistidine tag in HS3 and in addition by MTM (shown in red) at COOH-terminus in HS3M or at NH₂ terminus in HMS3. The recombinant proteins were purified under denaturing conditions and refolded in a Dulbecco Minimal Essential Medium (DMEM). Their intracellular function was analyzed in a mouse macrophage RAW 264.7 cell line.

Fig. 12

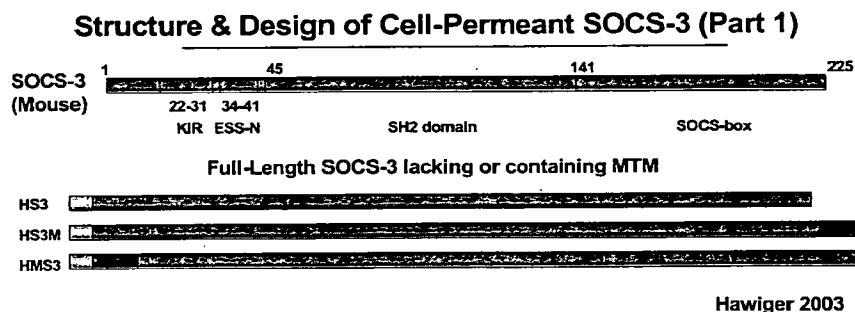


Fig. 13

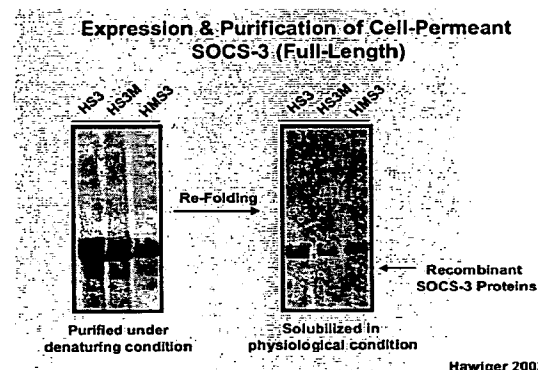
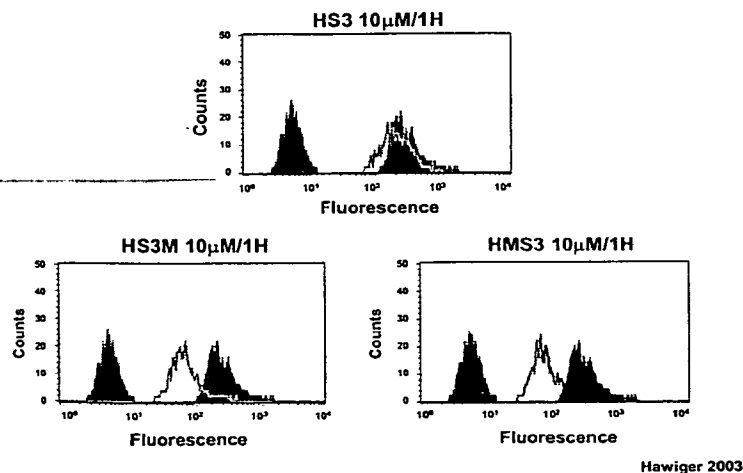


Fig. 14

Inhibition of STAT1 Phosphorylation by Cell-Permeant SOCS-3



C.4.2. Inhibition of STAT1 phosphorylation by cell-permeant SOCS3 protein. Mouse macrophage RAW 264.7 cell line was used to test the intracellular function of recombinant SOCS3 proteins that contain or lack the membrane translocating motif (MTM). Purified recombinant fusion SOCS-3 proteins (10 μ M) were added to cells for 1h then were stimulated with IFN- γ for 15 min (10 U/ml). The level of STAT1 phosphorylation in cell lysates was measured by cytometric bead array (BD CBA P-STAT1 Kit, BD Science, San Diego, USA). Median fluorescence intensity (MFI) was calculated by BD CBA software version 1.4 (BD Bioscience, San Diego, USA). Assays were conducted in duplicate or triplicate and repeated more than three times. As shown in Fig. 14, recombinant SOCS 3 protein without MTM (HS3) was inactive in terms of inhibition of STAT1 phosphorylation. In striking contrast to an MTM-

deficient protein, two recombinant SOCS 3 proteins that bear MTM at either end of the sequence had significant inhibitory effects on STAT1 phosphorylation.

C.4.2.1. Dose-Dependent Inhibition of STAT1 Phosphorylation by Cell-Permeant SOCS Proteins. Cells (RAW) were treated with purified, cell-permeant SOCS-3 proteins at 12, 6, and 3 μ M for 1h. Cells were then stimulated with IFN- γ (10 U/ml) for 15 min, washed with cold PBS, and lysed. The level of STAT1 phosphorylation was measured by cytometric bead array (BD CBA P-STAT1 Kit, BD Science, San Diego, USA). Intracellular inhibition of STAT1 phosphorylation was concentration dependent within 3 μ M to 12 μ M range of cell-permeant recombinant SOCS 3 proteins HS3M and HMS3 (results not shown). These results underscore the capacity of engineered SOCS 3 proteins to cross the plasma membrane via a membrane translocating motif (MTM) and interfere with STAT1 signaling.

D.4. Specific Aim 4: To Design and Test Cell-permeable Inhibitors of SEB-induced Signaling to the Nucleus

Rationale and Anticipated Results: Emboldened by our recent *in vivo* results with cell-permeable peptide antagonist of nuclear import of NF- κ B and other SRTFs (see Section C.1.3. Preliminary Studies and [83]), we plan to design new cell-permeable inhibitors that will target (i) TCR signaling, (ii) IFN γ -induced signaling to the nucleus, and (iii) transcription factor CIITA activation of MHC class II gene. Each of these intracellular targets offers the opportunity to regulate "overcharged" signaling induced by SAGs or proinflammatory cytokines (eg IFN γ). Thus, in this aim we will test the hypothesis that cell-permeable peptides and proteins transduced into NKT cells and T cells will disrupt intracellular signaling induced by SAGs. Moreover, we will test the hypothesis that IFN γ -induced signaling can be counteracted by cell-permeable SOCS-3 inhibitor and that expression of MHC Class II molecules can be similarly suppressed by a cell-permeable inhibitor IK. While we plan to study NF- κ B nuclear import antagonist in toxic shock induced by a second SAG, SPE-A, we propose to develop and test cell-permeable inhibitors that will block TCR receptor complex-mediated intracellular signaling as described in Specific Aim 1 (Section D.1.2.2). The cell-permeable peptide containing the zeta chain ITAM sequence LYQGLSTATKDTYDALHM will be rendered cell permeable by addition of the membrane translocating sequence [85]. The experiments in Aim 1 (Section D1.2.2) should provide information on the inhibitory potency of this cell-permeable peptide. Should the peptide exhibit solubility problems, we plan to produce and test a fusion protein containing the relevant functional cargo (see Aim 1).

The suppressor of cytokine signaling (SOCS) proteins constitute a growing family of cytokine-inducible negative regulators of cytokine signaling [151]. We plan to focus our efforts on the inhibitors of IFN γ signaling since this cytokine plays a key role in development of SEB-induced toxic shock [25] and in surface expression of the MHC class II molecules [29]. SOCS1 and SOCS3 but not SOCS2 inhibited the tyrosine phosphorylation and nuclear translocation of STAT1 in response to IFN γ stimulation [152]. The maximum inhibitory activities of STAT1 were observed at very low levels of SOCS protein expression. This bodes well for our planned experiment with a cell-permeable form of SOCS1 which exhibited a much stronger inhibitory activity than did SOCS3 [152].

The inhibitor IK acts as a negative regulator of transcription factor CIITA that controls both constitutive and inducible expression of MHC class II molecules [31]. We hypothesize that a cell-permeable form of IK will prevent inducible MHC class II expression thereby reducing the number of potential binding sites for SAGs.

The fusion protein will encompass polyhistidine tag, green fluorescent protein (GFP), functional "cargo" and the membrane translocating sequence (MTS) derived from the Kaposi FGF signal sequence. Murine NKT cell hybridoma and isolated murine liver NKT cells (see Section D.2.1.2) will be used to establish intracellular location of fusion protein and its potential inhibitory effect on cytokine production (TNF α , IFN γ , and IL-2) and NF- κ B activation as described in Section D.2.1.3. The cell-permeable zeta chain ITAM peptide or fusion protein will be tested *in vivo* in murine model of toxic shock induced by SEB in conjunction with D-Gal as a sensitizing agent (see Section C.1.3. Preliminary Studies and Section D.2.2 Research Design and Methods). The cell-permeable peptide or fusion protein will be injected intraperitoneally at concentrations of 700 μ g per 20 g mouse. These injections will be given 30 min before and 30, 90, 150, and 360 min after SEB. We anticipate a positive outcome of these experiments in terms of achieving cytokine suppression and reduced lethality. In such a case, SPE-A will be similarly tested. Inhibition of its lethal effect by cell-permeable peptide or fusion protein will extend the suppressive effect of the zeta chain ITAM motif-based cell permeable peptide to two distinct SAGs. However, if neither cell-permeable inhibitor exerts a suppressive effect on cytokine production and mouse lethality, we will analyze the effect of cell permeable antagonists of NF- κ B nuclear import.

As depicted in Fig. 2 the nuclear import of transcription factors constitutes a common step for distinct pathways signaling the nucleus. These pathways originate at the cell-surface of the TRC/CD3 complex and diverge downstream of the stress-responsive IKK signaling complex (NF- κ B), c-Jun kinase (AP-1), and calcineurin (NFAT)

checkpoints. The nuclear import complex comprised of importin/karyopherin α/β recognizes NF- κ B, AP-1 (c-fos and c-jun), and NFAT thereby serving as a common carrier for transcription factor delivery to the nucleus [43]. In Jurkat T cells, inhibition of importin α Rich 1 by intracellular delivery of cell-permeable SN50 peptide prevents translocation of activated transcription factors to the nucleus induced by phorbol ester and ionomycin [43]. We hypothesize that SPE A-induced nuclear import of transcription factors NF- κ B, AP-1 and NFAT in NKT cells and in T cells can be blocked by the cell-permeable SN50 peptide. Our approach should be particularly effective, because many genes encoding cytokines (e.g., IL-2, IFN γ , TNF α), cell-adhesion molecules, and procoagulant factors (e.g., tissue factor) are regulated by two or more transactivators [36, 43, 44]. We anticipate that SPE A-induced signaling to the nucleus will be inhibited by the SN50 peptide unless SPE-A-induced mechanisms differs from that induced by SEB (see Section C.1.2. Preliminary Studies).

The cell-permeable peptides and fusion proteins are designed to inhibit SAg-induced signaling in T cells and NKT cells at two distinct steps. First, at the TCR level by inhibiting ZAP 70 kinase known to be activated by SAg [153]. The use of cell-permeable peptides and fusion proteins to inhibit ZAP 70 kinase offers an approach to TCR-proximal signaling before its amplification by downstream transducers. Fortunately, even after SAg-induced signaling is set in motion and amplified, the inhibitors of NF- κ B nuclear import can stop it. Moreover, the nuclear import step targeted by cell-permeable peptide SN50 and cSN50 is involved in signaling mediated by four families of transcription factors, NF- κ B, AP1, NFAT, and STAT1 [43]. Thus, this TCR-distal signaling step is common for more than one transcription factor thereby offering a broad spectrum inhibition by cell-permeable peptides designed to block nuclear import in T cells, NKT cells, endothelial and monocyte/macrophage cells.

Another strategy proposed by us to inhibit SAg-induced toxic shock is focused on signaling by the proinflammatory cytokine IFN γ . This cytokine mediates SEB-induced toxic shock, because mice deficient in IFN γ receptor are refractory to SAg [25]. IFN γ induces expression of MHC class II molecules that bind SAg [27]. Therefore, we hypothesize that inhibition of IFN γ signaling and/or suppression of inducible expression of MHC class II molecules may prevent or attenuate SAg-induced toxic shock. Both signaling processes can be targeted using cell-permeable inhibitors we propose to develop and test in cultured cells and *in vivo* in murine model of SAg-induced toxic shock. IFN γ -induced MHC class II molecules expression is preceded by a positive regulation of CIITA gene expression [29]. The expression of MHC class II molecules and CIITA is negatively regulated by an intracellular inhibitor named IK (Inhibitor K562) [154]. The IK protein localized exclusively in cytoplasm suppresses the expression of CIITA thereby inhibiting expression of MHC Class II molecules.

We postulate that cell-permeable IK fusion protein will be capable of blocking MHC Class II induction by IFN γ . Moreover, we propose to use the IK fusion protein as cell-permeable inhibitor of class II MHC expression and ultimately as inhibitor of toxic shock induced by SAg. The IK fusion protein will be expressed in *E. coli*. The construct will contain polyhistidine tag for affinity purification and a second sequence motif representing membrane translocating sequence for transduction to cells *in vivo* and *in vitro* (see above the zeta chain ITAM motif fusion protein). The purified cell-permeable fusion KI protein will be first tested in IFN γ -inducible MHC Class II expressing cells (e.g. murine bone marrow-derived dendritic cells) and/or microvascular endothelial cells.

Inhibition of MHC Class II expression determined by FACS analysis will prompt us to test the IK fusion protein *in vivo* in murine model of SAg-induced toxic shock. The IK fusion protein will be administered ip into mice 24 h and 30 min before SEB. The expression of MHC class II molecules will be verified by FACS analysis of spleen B lymphocytes. The cell-permeable KI protein will be also administered after SAg injection (30, 90, 150, and 360 min) to establish whether suppressed expression of MHC class II molecules attenuates cytokine expression and lethal shock.

Methods

D.4.1. Synthesis and Purification of Cell-permeable Peptide Inhibitors: The peptides are routinely synthesized in the PI's laboratory using BOC chemistry as described [43]. The amino acid composition and mass of the synthesized peptides are routinely determined by an amino acid analyzer and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). The mutant peptides carrying the mutated functional domain will be synthesized and purified for control experiments. The SM peptide is cell-permeable but functionally inactive [43].

D.4.2. Expression and Purification of Cell Permeable Recombinant Fusion Protein: To assure rapid *in vivo* delivery of inhibitory peptides or proteins to cells stimulated by SAg and/or proinflammatory cytokines we will design recombinant fusion protein containing membrane translocation sequence (MTS), sequence encoding a functional cargo and a green fluorescent protein transduced to cells *in vitro* and *in vivo*. The construct will contain an amino-terminal affinity tag (polyHis) to facilitate purification of the recombinant protein and the MTS domain positioned at the carboxy terminus to allow easy and rapid transduction across plasma membrane [85]. Double-stranded oligonucleotides coding for a Kaposi FGF h region and for functional "cargo" will be cloned into pHAT-GFPuv vector from Clontech. Sequence and orientation will be confirmed by sequencing with reverse pHAT primer. Plasmids containing the construct will be transformed into the *E. Coli* expression host BL21(DE3)pLysS (Navagen) and grown to O.D. ~0.6 in LB broth containing 50 μ g/ml ampicillin at 37°. Cultures will be induced

with 1mM IPTG and incubated as before to O.D. 1.5-2.0 (~4h). Bacteria will be harvested and resuspended in Bacterial Protein Extraction Reagent (Pierce). After solubilization and centrifugation, the supernatant will be collected and mixed with an appropriate volume of 50% Ni-NTA agarose (Qiagen). Agarose beads will be washed with 50mM NaPO₄, 300 mM NaCl, 1 mM imidazole, pH 8.0. Proteins will be eluted using the same buffer containing 250 mM imidazole and fluorescence of GFP fusion will be examined on a 12% non-denaturing acrylamide gel. Peak protein fractions will be pooled and concentrated using a Centricon-10 filter (Amicon). After dialysis and determining protein concentration, the cell-permeable GFP fusion protein is examined for cell-transducing capacity by direct fluorescence in confocal laser scanning microscopy or indirect fluorescence with anti-GFP ab or anti-ITAM antibody. Similar constructs will be prepared for SOCS1 protein fusion and for IK protein fusion.

D.4.3. Measurement of Nuclear Import of NF- κ B Transcription Factors: The NKT cell hybridoma and the Jurkat cell line subclone J 77C120 responsive to the superantigen SEB will be used as described above. The SN50 and SM peptides (100 μ M) will be added 30 min before the addition of SAg (SEB or SPE A, 1-10 μ g see above). The cells will be incubated for 60 min (or shorter if the superantigen-induced nuclear import reaches maximum earlier). Electrophoretic mobility gel shift assays (as described above) and phosphoimaging will be performed to determine peptide-directed effects on SAg-induced nuclear import of NF- κ B, NF-AT, and AP-1.

Alternatively, we will test the effect of the SN50 peptide and its analogs on SPE A-induced proliferation of mouse spleen cells [140]. Briefly, mouse cells (5×10^5 /well) will be incubated with SPE A in 96-well microtiter plate for 72 hrs. They will be pulsed with [³H] thymidine to assess their proliferation in response to SPE A as compared to cells not challenged with SAg (background control).

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

G. LITERATURE CITED

1. Veach, R.A., Liu, D., Yao, S., Chen, Y., Liu, X.Y., Downs, S. and Hawiger, J. (2003) *J Biol Chem* **278**, 16774-16778.
2. Liu, D., Liu, X.Y., Robinson, D., Burnett, C., Jackson, C., Seele, L., Veach, R.A., Downs, S., Collins, R.D., Ballard, D.W. and Hawiger, J. (2004) *J Biol Chem* **279**, 19184-19191.
3. Liu, X.-Y., Robinson, D., Veach, R.A., Liu, D., Timmons, S., Collins, R.D. and Hawiger, J. (2004) *J Biol Chem* **279**, 16774-16778.
4. Liu, X.Y., Timmons, S., Lin, Y.Z. and Hawiger, J. (1996) *Proc Natl Acad Sci U S A* **93**, 11819-11824.
5. Zhang, L., Torgerson, T.R., Liu, X.Y., Timmons, S., Colosia, A.D., Hawiger, J. and Tam, J.P. (1997) *Proc Natl Acad Sci U S A* **95**, 9184-9189.
6. Hawiger, J. (2001) *Immunol Res* **23**, 99-109.
7. Yoshimura, A., Mori, H., Ohishi, M., Aki, D. and Hanada, T. (2003) *Curr Opin Immunol* **15**, 70-75.
8. Johnston, J.A. and O'Shea, J.J. (2003) *Nat Immunol* **4**, 507-509.
9. Kobayashi, K., Hernandez, L.D., Galan, J.E., Janeway, C.A., Jr., Medzhitov, R. and Flavell, R.A. (2001) *Cell* **104**, 191-202.
10. Baldwin, A.S., Jr. (1996) *Annu Rev Immunol* **14**, 649-683.
11. Ulevitch, R.J. and Tobias, P.S. (1995) *Annu Rev Immunol* **13**, 437-457.
12. Hawiger, J. (1994) *Lipopolysaccharide-induced signal transduction and gene transcription, in Endotoxin and the lungs*, K.L. Brigham, Editor. Marcel Dekker, Inc.: New York. p. 69-82.
13. Medzhitov, R. and Janeway, C., Jr. (2000) *N Engl J Med* **343**, 338-344.
14. Kerr, R., Stirling, D. and Ludlam, C.A. (2001) *Br J Haematol* **115**, 3-12.
15. Read, M.A., Cordle, S.R., Veach, R.A., Carlisle, C.D. and Hawiger, J. (1993) *Proc Natl Acad Sci U S A* **90**, 9887-9891.
16. O'Neill, L.A., Fitzgerald, K.A. and Bowie, A.G. (2003) *Trends Immunol* **24**, 286-290.
17. Dunne, A., Ejdeback, M., Ludidi, P.L., O'Neill, L.A. and Gay, N.J. (2003) *J Biol Chem* **278**, 41443-41451.
18. Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., Takeda, K. and Akira, S. (2003) *Science* **301**, 640-643.
19. Hoebe, K., Du, X., Georgel, P., Janssen, E., Tabet, K., Kim, S.O., Goode, J., Lin, P., Mann, N., Mu, S., Crozat, K., Sovath, S., Han, J. and Beutler, B. (2003) *Nature* **424**, 743-748.
20. Fitzgerald, K.A., Rowe, D.C., Barnes, B.J., Caffrey, D.R., Visintin, A., Latz, E., Monks, B., Pitha, P. and Golenbock, D.T. (2003) *J Exp Med* **198**, 1043-1055.
21. Melen, K., Fagerlund, R., Franke, J., Kohler, M., Kinnunen, L. and Julkunen, I. (2003) *J Biol Chem* **278**, 28193-28200.
22. McBride, K.M., Banninger, G., McDonald, C. and Reich, N.C. (2002) *Embo J* **21**, 1754-1763.
23. Torgerson, T.R., Colosia, A.D., Donahue, J.P., Lin, Y.Z. and Hawiger, J. (1998) *J Immunol* **161**, 6086-6092.
24. Gilmore, T.D. (1999) *Oncogene* **18**, 6842-6844.
25. Hawiger, J. (1994) *Brigham's monograph, in Lung Biology in Health and Disease*, C. Lenfant, Editor. Marcel Dekker, Inc.: New York.
26. Dinarello, C.A. (1996) *Curr Top Microbiol Immunol* **216**, 133-165.
27. Slack, J.L., Schooley, K., Bonnert, T.P., Mitcham, J.L., Qwarnstrom, E.E., Sims, J.E. and Dower, S. (2000) *J Biol Chem* **275**, 4670-4678.
28. Nawroth, P.P., Handley, D.A., Esmon, C.T. and Stern, D.M. (1986) *Proc Natl Acad Sci U S A* **83**, 3464-3468.
29. Zhang, Z. and Fuller, G.M. (2000) *Blood* **96**, 3466-3472.

30. Sironi, M., Breviario, F., Proserpio, P., Biondi, A., Vecchi, A., Van Damme, J., Dejana, E. and Mantovani, A. (1989) *J Immunol* **142**, 549-553.
31. Papanicolaou, D.A., Wilder, R.L., Manolagas, S.C. and Chrousos, G.P. (1998) *Ann Intern Med* **128**, 127-137.
32. Jones, S.A., Horiuchi, S., Topley, N., Yamamoto, N. and Fuller, G.M. (2001) *Faseb J* **15**, 43-58.
33. Boulanger, M.J., Bankovich, A.J., Kortemme, T., Baker, D. and Garcia, K.C. (2003) *Mol Cell* **12**, 577-589.
34. Heinrich, P.C., Behrmann, I., Muller-Newen, G., Schaper, F. and Graeve, L. (1998) *Biochem J* **334**, 293-314.
35. Chen, G. and Goeddel, D.V. (2002) *Science* **296**, 1634-1635.
36. Ihle, J.N. (1996) *Cell* **84**, 331-334.
37. Levy, D.E. and Darnell, J.E., Jr. (2002) *Nat Rev Mol Cell Biol* **3**, 651-662.
38. van der Poll, T., Buller, H.R., ten Cate, H., Wortel, C.H., Bauer, K.A., van Deventer, S.J., Hack, C.E., Sauerwein, H.P., Rosenberg, R.D. and ten Cate, J.W. (1990) *N Engl J Med* **322**, 1622-1627.
39. Hesse, D.G., Tracey, K.J., Fong, Y., Manogue, K.R., Palladino, M.A., Jr., Cerami, A., Shires, G.T. and Lowry, S.F. (1988) *Surg Gynecol Obstet* **166**, 147-153.
40. Krebs, D.L. and Hilton, D.J. (2000) *J Cell Sci* **113**, 2813-2819.
41. Croker, B.A., Krebs, D.L., Zhang, J.G., Wormald, S., Willson, T.A., Stanley, E.G., Robb, L., Greenhalgh, C.J., Forster, I., Clausen, B.E., Nicola, N.A., Metcalf, D., Hilton, D.J., Roberts, A.W. and Alexander, W.S. (2003) *Nat Immunol* **4**, 540-545.
42. Lang, R., Pauleau, A.L., Parganas, E., Takahashi, Y., Mages, J., Ihle, J.N., Rutschman, R. and Murray, P.J. (2003) *Nat Immunol* **4**, 546-550.
43. Yasukawa, H., Ohishi, M., Mori, H., Murakami, M., Chinen, T., Aki, D., Hanada, T., Takeda, K., Aki, S., Hoshijima, M., Hirano, T., Chien, K.R. and Yoshimura, A. (2003) *Nat Immunol* **4**, 551-556.
44. Ratnoff, O.D. (1969) *Adv Immunol* **10**, 145-227.
45. Henn, V., Slupsky, J.R., Grafe, M., Anagnostopoulos, I., Forster, R., Muller-Berghaus, G. and Kroczel, R.A. (1998) *Nature* **391**, 591-594.
46. Andre, P., Prasad, K.S., Denis, C.V., He, M., Papalia, J.M., Hynes, R.O., Phillips, D.R. and Wagner, D.D. (2002) *Nat Med* **8**, 247-252.
47. Nannizzi-Alaimo, L., Rubenstein, M.H., Alves, V.L., Leong, G.Y., Phillips, D.R. and Gold, H.K. (2000) *Circulation* **105**, 2849-2854.
48. Heeschen, C., Dimmeler, S., Hamm, C.W., van den Brand, M.J., Boersma, E., Zeiher, A.M. and Simoons-Smit, A.M. (2003) *N Engl J Med* **348**, 1104-1111.
49. Nannizzi-Alaimo, L., Alves, V.L. and Phillips, D.R. (2003) *Circulation* **107**, 1123-1128.
50. Hawiger, J. (2002) *Peptide/Protein Delivery*, in *Wiley Encyclopedia of Molecular Medicine*. John Wiley & Sons, Inc. p. 2435-2438.
51. Jo, D., Nashabi, A., Doxsee, C., Lin, Q., Unutmaz, D., Chen, J. and Ruley, H.E. (2001) *Nat Biotechnol* **19**, 929-933.
52. Schwarze, S.R. and Dowdy, S.F. (2000) *Trends Pharmacol Sci* **21**, 45-48.
53. Huang, S. (2000) *Nat Biotechnol* **18**, 471-472.
54. Verma, R., Boleti, E. and George, A.J. (1998) *J Immunol Methods* **216**, 165-181.
55. Clare, J.J., Rayment, F.B., Ballantine, S.P., Sreekrishna, K. and Romanos, M.A. (1991) *Biotechnology (N Y)* **9**, 455-460.
56. Chappell, J.D., Duong, J.L., Wright, B.W. and Dermody, T.S. (2000) *J Virol* **74**, 8472-8479.
57. Hawiger, J. (1999) *Curr Opin Chem Biol* **3**, 89-94.
58. Liu, X.Y., Robinson, D., Veach, R.A., Liu, D., Timmons, S., Collins, R.D. and Hawiger, J. (2000) *J Biol Chem* **275**, 16774-16778.
59. Martin, G.S., Mannino, D.M., Eaton, S. and Moss, M. (2003) *N Engl J Med* **348**, 1546-1554.
60. Hawiger, J. (2003) *Blood* **102**, 1940-1941.

61. Hawiger, J., Hawiger, A. and Timmons, S. (1975) *Nature* **256**, 125-127
62. Hawiger, J., Parkinson, S. and Timmons, S. (1980) *Nature* **283**, 195-197.
63. Timmons, S., Kloczewiak, M. and Hawiger, J. (1984) *Proc Natl Acad Sci U S A* **81**, 4935-4939.
64. Kloczewiak, M., Timmons, S., Lukas, T.J. and Hawiger, J. (1984) *Biochemistry* **23**, 1767
65. Cordle, S.R., Donald, R., Read, M.A. and Hawiger, J. (1993) *J Biol Chem* **268**, 11803-11810.
66. Donald, R., Ballard, D.W. and Hawiger, J. (1995) *J Biol Chem* **270**, 9-12.
67. Lin, Y.Z., Yao, S.Y., Veach, R.A., Torgerson, T.R. and Hawiger, J. (1995) *J Biol Chem* **270**, 14255-14258.
68. Torgerson, T.R., Colosia, A.D., Donahue, J.P., Lin, Y.Z. and Hawiger, J. (1998) *J Immunol* **161**, 6084-6092.
69. Galanos, C., Freudenberg, M.A. and Reutter, W. (1979) *Proc Natl Acad Sci U S A* **76**, 5939-5943
70. Miethke, T., Wahl, C., Heeg, K., Echtenacher, B., Krammer, P.H. and Wagner, H. (1992) *J Exp Med* **175**, 91-98
71. Car, B.D., Eng, V.M., Schnyder, B., Ozmen, L., Huang, S., Gallay, P., Heumann, D., Aguet, M. and Ryffel, B. (1994) *J. Exp. Med.* **179**, 1437-1444
72. Ozato, K., Tsujimura, H. and Tamura, T. (2002) *Biotechniques Suppl*, 66-68, 70, 72 passim.
73. Heinrich, P.C., Castell, J.V. and Andus, T. (1990) *Biochem J* **265**, 621-636.
74. Shouda, T., Yoshida, T., Hanada, T., Wakioka, T., Oishi, M., Miyoshi, K., Komiya, S., Kosai, K., Hanakawa, Y., Hashimoto, K., Nagata, K. and Yoshimura, A. (2001) *J Clin Invest* **108**, 1781-1788.
75. Bertolino, P., Trescol-Biemont, M.C. and Rabourdin-Combe, C. (1998) *Eur J Immunol* **28**, 221-236.
76. Brysha, M., Zhang, J.G., Bertolino, P., Corbin, J.E., Alexander, W.S., Nicola, N.A., Hilton, D.J. and Starr, R. (2001) *J Biol Chem* **276**, 22086-22089.
77. Krebs, D.L. and Hilton, D.J. (2001) *Stem Cells* **19**, 378-387.
78. Lehmann, U., Schmitz, J., Weissenbach, M., Sobota, R.M., Hortner, M., Friederichs, K., Behrmann, I., Tsiaris, W., Sasaki, A., Schneider-Mergener, J., Yoshimura, A., Neel, B.G., Heinrich, P.C. and Schaper, F. (2003) *J Biol Chem* **278**, 661-671.
79. Jalbert, L.R., Rosen, E.D., Moons, L., Chan, J.C., Carmeliet, P., Collen, D. and Castellino, F.J. (1998) *J Clin Invest* **102**, 1481-1488.
80. Tsakiris, D.A., Scudder, L., Hodivala-Dilke, K., Hynes, R.O. and Collier, B.S. (1999) *Thromb Haemost* **81**, 177-188.
81. Hawiger, J., Niewiarowski, S., Gurewich, V. and Thomas, D.P. (1970) *J Lab Clin Med* **75**, 93-108.
82. Ravanat, C., Freund, M., Dol, F., Cadroy, Y., Roussi, J., Incardona, F., Maffrand, J.P., Boneu, B., Drouet, L., Legrand, C. and et al. (1995) *Blood Coagul Fibrinolysis* **6**, 446-455.
83. Weiler-Guettler, H., Christie, P.D., Beeler, D.L., Healy, A.M., Hancock, W.W., Rayburn, H., Edelberg, J.M. and Rosenberg, R.D. (1998) *J Clin Invest* **101**, 1983-1991.
84. Christie, P.D., Edelberg, J.M., Picard, M.H., Foulkes, A.S., Mamuya, W., Weiler-Guettler, H., Rubin, R.H., Gilbert, P. and Rosenberg, R.D. (1999) *J Clin Invest* **104**, 533-539.
85. Hawiger, J. and Timmons, S. (1992) *Methods in Enzymology* **215**, 228-243
86. Collier, B.S., Peerschke, E.I., Scudder, L.E. and Sullivan, C.A. (1983) *J Clin Invest* **72**, 325-338.
87. Huang, T.F., Holt, J.C., Lukasiewicz, H. and Niewiarowski, S. (1987) *J Biol Chem* **262**, 16157-16163.
88. Donahue, J.P., Patel, H., Anderson, W.F. and Hawiger, J. (1994) *Proceedings of the National Academy of Sciences of the United States of America* **91**, 12178-12182
89. Quinn, M.J., Plow, E.F. and Topol, E.J. (2002) *Circulation* **106**, 379-385.
90. May, A.E., Kalsch, T., Massberg, S., Herouy, Y., Schmidt, R. and Gawaz, M. (2002) *Circulation* **106**, 2111-2117.
91. Prasad, K.S., Andre, P., He, M., Bao, M., Manganello, J. and Phillips, D.R. (2003) *Proc Natl Acad Sci U S A* **100**, 12367-12371.
92. Timmons, S. and Hawiger, J. (1989) *Methods in Enzymology* **169**, 11-21
93. O'Neill, L.A. (2003) *Curr Opin Pharmacol* **3**, 396-403.
94. Radons, J., Gabler, S., Wesche, H., Korherr, C., Hofmeister, R. and Falk, W. (2002) *J Biol Chem* **277**, 16456-16463.
95. Kawai, T., Adachi, O., Ogawa, T., Takeda, K. and Akira, S. (1999) *Immunity* **11**, 115-122
96. Yamamoto, M., Sato, S., Mori, K., Hoshino, K., Takeuchi, O., Takeda, K. and Akira, S. (2002) *J Immunol* **169**, 6668-6672.
97. Sato, M., Suemori, H., Hata, N., Asagiri, M., Ogasawara, K., Nakao, K., Nakaya, T., Katsuki, M., Noguchi, S., Tanaka, N. and Taniguchi, T. (2000) *Immunity* **13**, 539-548.

What is claimed is:

1. A method for inhibiting a cytokine-induced response in a cell,
comprising administering to the cell a complex comprising a cell
5 membrane permeable hydrophobic region of a signal peptide linked to a
polypeptide comprising an amino acid sequence encoding a SOCS
protein or a fragment thereof.
2. The method of claim 1, wherein the cell membrane permeable
hydrophobic region of a signal peptide is a membrane translocating
10 motif from FGF-4
3. The method of claim 1, wherein the SOCS protein is a SOCS 1 protein.
4. The method of claim 1, wherein the SOCS protein is a SOC 3 protein.
5. A method of inhibiting a cytokine-induced response in a cell in a
subject, comprising administering to the cell in a subject a complex
15 comprising a cell membrane permeable hydrophobic region of a signal
peptide linked to a polypeptide comprising an amino acid sequence
encoding a SOCS protein or a fragment thereof.
6. The method of claim 1, wherein the cell membrane permeable
hydrophobic region of a signal peptide is a membrane translocating
20 motif from FGF-4
7. The method of claim 1, wherein the SOCS protein is a SOCS 1 protein.
8. The method of claim 1, wherein the SOCS protein is a SOC 3 protein.

SEQUENCE LISTING

SEQ ID NO: 1 (His tag)

MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySer

SEQ ID NO: 2 (MTM)

AlaAlaValLeuLeuProValLeuLeuAlaAlaPro

SEQ ID NO: 3 (full-length SOCS 1)

MetValAlaArgAsnGlnValAlaAlaAspAsnAlaIleSerProAlaAlaGluProArgArgArgSerGlu
ProSerSerSerSerSerSerSerProAlaAlaProValArgProArgProCysProAlaValProAlaPro
AlaProGlyAspThrHisPheArgThrPheArgSerHisSerAspTyrArgArgIleThrArgThrSerAla
LeuLeuAspAlaCysGlyPheTyrTrpGlyProLeuSerValHisGlyAlaHisGluArgLeuArgAlaGlu
ProValGlyThrPheLeuValArgAspSerArgGlnArgAsnCysPhePheAlaLeuSerValLysMET
AlaSerGlyProThrSerIleArgValHisPheGlnAlaGlyArgPheHisLeuAspGlySerArgGluThr
PheAspCysLeuPheGluLeuLeuGluHisTyrValAlaAlaProArgArgMETLeuGlyAlaProLeu
ArgGlnArgArgValArgProLeuGlnGluLeuCysArgGlnArgIleValAlaAlaValGlyArgGluAsn
LeuAlaArgIleProLeuAsnProValLeuArgAspTyrLeuSerSerPheProPheGlnIle

SEQ ID NO: 4 (full-length SOCS 3)

MetValThrHisSerLysPheProAlaAlaGlyMETSerArgProLeuAspThrSerLeuArgLeuLysThr
PheSerSerLysSerGluTyrGlnLeuValValAsnAlaValArgLysLeuGlnGluSerGlyPheTyrTrp
SerAlaValThrGlyGlyGluAlaAsnLeuLeuLeuSerAlaGluProAlaGlyThrPheLeuIleArgAsp
SerSerAspGlnArgHisPhePheThrLeuSerValLysThrGlnSerGlyThrLysAsnLeuArgIleGln
CysGluGlyGlySerPheSerLeuGlnSerAspProArgSerThrGlnProValProArgPheAspCysVal
LeuLysLeuValHisHisTyrMETProProProGlyThrProSerPheSerLeuProProThrGluProSer
SerGluValProGluGlnProProAlaGlnAlaLeuProGlySerThrProLysArgAlaTyrTyrIleTyrSer
GlyGlyGluLysIleProLeuValLeuSerArgProLeuSerSerAsnValAlaThrLeuGlnHisLeuCys
ArgLysThrValAsnGlyHisLeuAspSerTyrGluLysValThrGlnLeuProGlyProIleArgGluPhe
LeuAspGlnTyrAspAlaProLeu

SEQ ID NO: 5 (His-SOCS1-MTM)

MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySer MetValAlaArgAsn
GlnValAlaAlaAspAsnAlaIleSerProAlaAlaGluProArgArgArgSerGluProSerSerSerSer
SerSerSerProAlaAlaProValArgProArgProCysProAlaValProAlaProAlaProGlyAspThrHis
PheArgThrPheArgSerHisSerAspTyrArgArgIleThrArgThrSerAlaLeuLeuAspAlaCysGly
PheTyrTrpGlyProLeuSerValHisGlyAlaHisGluArgLeuArgAlaGluProValGlyThrPheLeu
ValArgAspSerArgGlnArgAsnCysPhePheAlaLeuSerValLysMETAlaSerGlyProThrSerIle
ArgValHisPheGlnAlaGlyArgPheHisLeuAspGlySerArgGluThrPheAspCysLeuPheGlu
LeuLeuGluHisTyrValAlaAlaProArgArgMETLeuGlyAlaProLeuArgGlnArgArgValArg
ProLeuGlnGluLeuCysArgGlnArgIleValAlaAlaValGlyArgGluAsnLeuAlaArgIleProLeu
AsnProValLeuArgAspTyrLeuSerSerPheProPheGlnIle AlaAlaValLeuLeuProValLeuLeu
AlaAlaPro

SEQ ID NO: 6 (His-MTM-SOCS1)

MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerAlaAlaValLeuLeuPro
ValLeuLeuAlaAlaProMetValAlaArgAsnGlnValAlaAlaAspAsnAlaIleSerProAlaAlaGlu
ProArgArgArgSerGluProSerSerSerSerSerSerSerProAlaAlaProValArgProArgProCys
ProAlaValProAlaProAlaProGlyAspThrHisPheArgThrPheArgSerHisSerAspTyrArgArg
IleThrArgThrSerAlaLeuLeuAspAlaCysGlyPheTyrTrpGlyProLeuSerValHisGlyAlaHis
GluArgLeuArgAlaGluProValGlyThrPheLeuValArgAspSerArgGlnArgAsnCysPhePhe
AlaLeuSerValLysMETAlaSerGlyProThrSerIleArgValHisPheGlnAlaGlyArgPheHisLeu
AspGlySerArgGluThrPheAspCysLeuPheGluLeuLeuGluHisTyrValAlaAlaProArgArg
METLeuGlyAlaProLeuArgGlnArgArgValArgProLeuGlnGluLeuCysArgGlnArgIleVal
AlaAlaValGlyArgGluAsnLeuAlaArgIleProLeuAsnProValLeuArgAspTyrLeuSerSerPhe
ProPheGlnIle

SEQ ID NO: 7 (His-SOCS3)

MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerMetValThrHisSerLys
PheProAlaAlaGlyMETSerArgProLeuAspThrSerLeuArgLeuLysThrPheSerSerLysSerGlu
TyrGlnLeuValValAsnAlaValArgLysLeuGlnGluSerGlyPheTyrTrpSerAlaValThrGlyGly
GluAlaAsnLeuLeuLeuSerAlaGluProAlaGlyThrPheLeuIleArgAspSerSerAspGlnArgHis
PhePheThrLeuSerValLysThrGlnSerGlyThrLysAsnLeuArgIleGlnCysGluGlyGlySerPhe
SerLeuGlnSerAspProArgSerThrGlnProValProArgPheAspCysValLeuLysLeuValHisHis
TyrMETProProProGlyThrProSerPheSerLeuProProThrGluProSerSerGluValProGluGln
ProProAlaGlnAlaLeuProGlySerThrProLysArgAlaTyrTyrIleTyrSerGlyGlyGluLysIlePro
LeuValLeuSerArgProLeuSerSerAsnValAlaThrLeuGlnHisLeuCysArgLysThrValAsnGly
HisLeuAspSerTyrGluLysValThrGlnLeuProGlyProIleArgGluPheLeuAspGlnTyrAspAla
ProLeu

SEQ ID NO: 8 (His-SOCS3-MTM)

MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerMetValThrHisSerLys
PheProAlaAlaGlyMETSerArgProLeuAspThrSerLeuArgLeuLysThrPheSerSerLysSerGlu
TyrGlnLeuValValAsnAlaValArgLysLeuGlnGluSerGlyPheTyrTrpSerAlaValThrGlyGly
GluAlaAsnLeuLeuLeuSerAlaGluProAlaGlyThrPheLeuIleArgAspSerSerAspGlnArgHis
PhePheThrLeuSerValLysThrGlnSerGlyThrLysAsnLeuArgIleGlnCysGluGlyGlySerPhe
SerLeuGlnSerAspProArgSerThrGlnProValProArgPheAspCysValLeuLysLeuValHisHis
TyrMETProProProGlyThrProSerPheSerLeuProProThrGluProSerSerGluValProGluGln
ProProAlaGlnAlaLeuProGlySerThrProLysArgAlaTyrTyrIleTyrSerGlyGlyGluLysIlePro
LeuValLeuSerArgProLeuSerSerAsnValAlaThrLeuGlnHisLeuCysArgLysThrValAsnGly
HisLeuAspSerTyrGluLysValThrGlnLeuProGlyProIleArgGluPheLeuAspGlnTyrAspAla
ProLeuAlaAlaValLeuLeuProValLeuLeuAlaAlaPro

SEQ ID NO: 9 (His-MTM-SOC3)

MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerAlaAlaValLeuLeuPro
ValLeuLeuAlaAlaProMetValThrHisSerLysPheProAlaAlaGlyMETSerArgProLeuAspThr
SerLeuArgLeuLysThrPheSerSerLysSerGluTyrGlnLeuValValAsnAlaValArgLysLeuGln
GluSerGlyPheTyrTrpSerAlaValThrGlyGlyGluAlaAsnLeuLeuLeuSerAlaGluProAlaGly
ThrPheLeuIleArgAspSerSerAspGlnArgHisPhePheThrLeuSerValLysThrGlnSerGlyThr
LysAsnLeuArgIleGlnCysGluGlyGlySerPheSerLeuGlnSerAspProArgSerThrGlnProVal
ProArgPheAspCysValLeuLysLeuValHisHisTyrMETProProProGlyThrProSerPheSerLeu
ProProThrGluProSerSerGluValProGluGlnProProAlaGlnAlaLeuProGlySerThrProLysArg
AlaTyrTyrIleTyrSerGlyGlyGluLysIleProLeuValLeuSerArgProLeuSerSerAsnValAlaThr
LeuGlnHisLeuCysArgLysThrValAsnGlyHisLeuAspSerTyrGluLysValThrGlnLeuProGly
ProIleArgGluPheLeuAspGlnTyrAspAlaProLeu